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(54) Title: PEPTIDE ANALOGUES CONTAINING A 7-MEMBERED LACTAM RING			
(57) Abstract The invention concerns pharmaceutically useful peptide derivatives of the formula (I), $P-R^1-R^2-R^3-R^4$, in which P, R ¹ , R ² , R ³ , and R ⁴ have the various meanings defined herein, and their pharmaceutically acceptable salts, and pharmaceutical compositions containing them. The novel peptide derivatives are of value in treating MHC class II dependent T-cell mediated autoimmune or inflammatory diseases, such as rheumatoid arthritis. The invention further concerns processes for the manufacture of the novel peptide derivatives and the use of the compounds in medical treatment.			

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PEPTIDE ANALOGUES CONTAINING A 7-MEMBERED LACTAM RING

The present invention relates to certain novel peptide compounds which possess pharmacologically useful properties for use in treating autoimmune diseases or medical conditions, such as rheumatoid arthritis and other MHC class II dependent T-cell mediated diseases. The invention also includes pharmaceutical compositions of the novel peptide compounds, processes for their manufacture, and their use in treating one or more of the aforementioned diseases or medical conditions and in the production of novel pharmaceuticals for use in such medical treatments.

Stimulation of the human immune response is dependent on the recognition of protein antigens by T-cells. However T-cells cannot respond to antigen alone and are only triggered by antigen when it is complexed with major histocompatibility complex (MHC) molecules on the surface of an antigen presenting cell, such as a B cell, macrophage or dendritic cell.

MHC class I molecules elicit a T-killer cell response which results in the destruction of the cell bearing the antigen. MHC class II molecules elicit a T-helper cell response which controls the expansion and maturation of selected B cells (i.e. generation of antigen-specific antibodies) and activation of macrophages.

A critical requirement of the immune system is the ability to differentiate between "self" and "non-self" (i.e. foreign) antigens. This discrimination is required to enable the immune system to mount a response to invading foreign pathogens whilst maintaining tolerance to self-proteins and thereby preventing damage to normal tissues. An autoimmune disease results when self-tolerance breaks down allowing the immune system to react against self-tissues such as the joints in rheumatoid arthritis. It is thought that the maintenance of tolerance and thus avoidance of autoimmune disease is critically dependent on the function of MHC molecules.

The observation that many autoimmune diseases are linked to the inheritance of particular MHC alleles suggests a key role for MHC molecules in the pathogenesis of autoimmune disease. For instance multiple sclerosis is linked to the inheritance of HLA-DR2, insulin dependent diabetes mellitus to HLA-DR3 and/or HLA-DR4 and Hashimoto's thyroiditis to HLA-DR5. In particular, an especially strong association exists between predisposition to development of the chronic inflammatory joint disease rheumatoid arthritis and the inheritance of HLA-DR4Dw4 and/or HLA-DR4w14 and/or HLA-DR1. It is thought

that the autoimmune disease associated MHC molecules bind to certain self-antigens and present them to T-cells thus stimulating an autoimmune response. Other peptides which can bind to the autoimmune associated MHC molecules and/or either prevent the binding or displace already bound self-antigens and/or which inhibit T-cell activation (especially the activity of pathogenic T-cells (e.g. Th 1 cells)) and/or which increase the activity of protective T-cells (e.g. Th 2 cells) or peptides which interact with MHC molecules by an alternative mechanism of action so as to prevent or modify stimulation of an autoimmune response mediated via said MHC molecules, may specifically suppress an autoimmune response.

An agent of this kind would offer therapy for the autoimmune disease whilst avoiding general suppression of the immune system, thus limiting deleterious side-effects. This kind of profile would have significant advantages over current therapy for diseases such as rheumatoid arthritis. It is contemporary practice to treat rheumatoid arthritis initially with symptom relief agents such as NSAIDs, which do not have any beneficial effect on disease progression and are often associated with unwanted side-effects. Treatment of more severe disease relies on the use of the so-called second-line agents. Often these are general cytotoxic compounds which are of limited efficacy and can cause severe toxicity problems. A rationally based, disease modifying agent, without associated non-specific cytotoxicity, would therefore offer significant benefits in the treatment of rheumatoid arthritis.

Peptides are disclosed in International Patent Application, Publication No's. WO 92/02543, WO 93/05011 and WO 95/07707 which bind to MHC molecules and inhibit T-cell activation.

Although a number of peptides have been discovered which inhibit HLA-DR restricted T-cell activation by binding to HLA-DR molecules, there is a continuing need for alternative compounds which bind to such molecules and/or either prevent the binding or displace already bound self-antigens and/or inhibit T-cell activation and/or increase the activity of protective T-cells, or which interact with MHC molecules by an alternative mechanism of action, so as to prevent or modify stimulation of an autoimmune response that causes a disease or condition referred to above.

We have discovered that the peptide compounds of the present invention (set out below) surprisingly possess such pharmacologically useful properties and this is a basis for the present invention.

According to one aspect of the invention there is provided a peptide compound of the formula I (set out hereinafter)

wherein

- P is a hydrophobic residue; R^1 is a sequence of 5 L-amino acids and R^3 is a single L-amino acid; or R^1 is a sequence of 3 L-amino acids and R^3 is a sequence of 3 L-amino acids;
- R^2 is a group of the formula II (set out hereinafter) in which R_a is selected from hydrogen and (1-4C)alkyl; and
- R^4 is OH, NH_2 or $NRcRd$ wherein Rc is selected from (1-4C)alkyl, 2-carbamoylcyclopentyl, 2-pyridylmethyl, 4-carbamoylcyclohexyl, 4-carbamoylcyclohexylmethyl, 3-carbamoylphenyl, 4-carbamoylphenyl, 4-(carbamoylmethyl)phenyl, 4-(carboxymethyl)phenyl, 2-morpholinoethyl and a group of the formula $-A^1-G^1$ in which A^1 is (3-7C)alkylene or A^1 is selected from
- (1) a group of the formula $-A^2-B^2-$ in which A^2 is *p*-phenylene or 1,4-cyclohexylene and B^2 is (1-4C)alkylene or A^2 is methylene and B^2 is *p*-phenylene or 1,4-cyclohexylene; and
- (2) a group of the formula $-A^3-B^3-C^3-$ in which A^3 is methylene, B^3 is *p*-phenylene or 1,4-cyclohexylene and C^3 is (1-3C)alkylene; and
- G^1 is a group of the formula $-N=C[N(Rp)_2]_2$ in which each Rp is independently selected from hydrogen, methyl, ethyl and propyl; and Rd is hydrogen or (1-4C)alkyl; or
- R^4 is 1-piperazinyl, 4-methyl-1-piperazinyl, 4-amidino-1-piperazinyl, 4-(2-(2-hydroxyethoxy)ethyl)-1-piperazinyl, 1-piperidyl or 4-substituted-1-piperidyl wherein the 4-substituent is selected from carboxy, carbamoyl, N-(2-aminoethyl)carbamoyl and N-(4-aminobutyl)carbamoyl; or
- R^4 is a sequence of 1 to 6 amino acids or an amide thereof; or a pharmaceutically acceptable salt thereof.

- It is to be understood that an amino acid of R^4 may independently have the D- or L-stereochemistry. Furthermore, when R^4 is defined as hydroxy (OH), this will be understood to be the hydroxy group of the C-terminal amino acid of R^3 . Similarly where R^4 is defined as NH_2 , $NRcRd$, piperazinyl, piperidyl, etc., this means that the hydroxy group of the C-terminal amino acid of R^3 is replaced by such a group. It is also to be understood that where an amino acid is referred to this means an alpha-amino acid. It is also to be understood that when an L-amino acid is referred to this also includes amino acids such as Gly, 2,2-diethylGly, aza-alanine and aza-glycine which have no chiral carbon atom. It is further to be understood that

generic terms such as "alkyl" include both straight and branched chain variants when the carbon numbers permit. The same convention applies to other radicals.

It is well known in the art that compounds having a chiral centre may exist in the form of a racemate (or a mixture of diastereoisomers where there is more than one chiral centre) or as an optically active enantiomer or diastereoisomer. It is also well known in the art that a particular biological activity associated with a racemic or diastereomeric mixture may result largely or solely from a single optically active isomer. It will therefore be understood that the invention concerns any form of a chemical compound of formula I which possesses the aforementioned pharmaceutically useful properties. It is well known in the art how to obtain a single optically active isomer, for example by separation from a racemic or diastereomeric mixture containing the isomer using conventional techniques, such as chromatography, or by chiral synthesis using an appropriate optically active starting material or intermediate, as exemplified herein. It is also well known in the art how to determine the pharmacological properties of such racemic or diastereomeric mixtures, and the individual optically active isomers, for example by using the assays described herein. The person skilled in the art is therefore easily able to obtain the particular isomers of the chemical compounds of formula I having the beneficial pharmacological properties referred to herein.

It is also to be understood that the present invention also encompasses any polymorphic form, any tautomer or any solvate, or any mixture thereof, of a peptide compound of formula I which possesses the beneficial pharmacological properties referred to herein.

Suitable independent values for the α -amino acids comprising R^1 and R^3 generally include, for example, the 20 naturally occurring amino acids encoded by the genetic code, particularly alanine (Ala), glutamic acid (Glu), glycine (Gly), histidine (His), isoleucine (Ile), lysine (Lys), asparagine (Asn), glutamine (Gln), arginine (Arg), threonine (Thr) and valine (Val). Amino acids such as sarcosine (Sar), 3,3,3-trifluoroalanine, 2,2-diethylglycine, 2,3-diaminopropanoic acid (Dap), 2,4-diaminobutanoic acid (Dab), 2-aminobutanoic acid (Abu), homoarginine, homophenylalanine, trans-4-hydroxyproline (Hyp), aza-alanine [$H_2N-N(CH_3)-COOH$; Azala], aza-glycine [$H_2N-NH-COOH$; Azgly], 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (Tic), octahydroindole-2-carboxylic acid (Oic), decahydroisoquinoline-3-carboxylic acid (Dic) are also suitable. (Where Dic is referred to this means the forms in which the ring-junctions both have the R configuration or both have

the S configuration.) Corresponding N²-methylated amino acids may also be used, as well as corresponding amino acids in which a free side-chain carboxylic acid function is esterified (for example as an (1-6C)alkyl or benzyl ester) and a free side-chain amino group is alkylated, (for example, methylated), acetylated or converted to a carbamate (for example, an alkyl (such as methyl or ethyl), phenyl or benzyl carbamate). Other suitable values for R¹ and R³ include, 5 for example, 2-substituted glycine in which the 2-substituent is a group of the formula - (CH₂)_sNH₂ wherein s is 1 to 3, or a group of the formula -(CH₂)_pN(Re)₃⁺.X⁻ wherein p is 2 to 4 and X⁻ is a counter ion (such as acetate, trifluoroacetate, hydroxide or chloride), or a group of the formula -(CH₂)_qN(Re)₂ wherein q is 0 to 4 or a group of the formula 10 -(CH₂)_rN=C[N(Re)₂]₂ wherein r is 1 to 4, wherein in which last three groups each Re is independently selected from hydrogen and (1-4C)alkyl (such as methyl or ethyl).

A value for R¹ of particular interest when it is a sequence of 5 amino acids includes, for example, a sequence in which the fifth amino acid (as read from left to right) is Val or Thr and a sequence in which the fourth and fifth amino acids is Lys-Val, Arg-Val, Lys-Thr, Arg-Thr, Ala-Val or Ala-Thr. 15

A particular value for R¹ when it is a sequence of 5 amino acids includes, for example, Ala-Ala-Ala-Lys-Val, Ala-Lys-Ala-Ala-Val, Ala-Ala-Ala-Arg-Val, Ala-Arg-Ala-Ala-Val, Ala-Lys-Ala-Lys-Val, Ala-Arg-Ala-Arg-Val, Ala-Arg-Ala-Lys-Val, Ala-Lys-Ala-Arg-Val, Ile-Ala-Ala-Arg-Thr, Arg-Ala-Ala-Ala-Val, Arg-Ala-Ala-Ala-Thr, Ala-Ala-Ala-Arg-Thr, 20 Ala-Arg-Ala-Arg-Thr, Ala-Ile-Ala-Arg-Val, Ala-Arg-Ala-His-Val, Ala-Arg-Ala-Ala-Thr, Ala-Ala-Asn-Arg-Val or X-Ala-Ala-Ala-Thr where X is -NH.CH[CH₂NH(=NH).NH₂].CO- (hereinafter referred to as "Gap"), or -NH.CH(CH₂N=C[N(CH₃)₂]).CO- (hereinafter referred to as "GapMe₄"), of which Ala-Ala-Ala-Lys-Val, Arg-Ala-Ala-Ala-Val, Arg-Ala-Ala-Ala-Thr, Ala-Ala-Ala-Arg-Thr, Ala-Arg-Ala-Arg-Thr, Gap-Ala-Ala-Ala-Thr and GapMe₄-Ala- 25 Ala-Ala-Thr are preferred. Arg-Ala-Ala-Ala-Val and Arg-Ala-Ala-Ala-Thr are particularly preferred.

A value for R¹ of particular interest when it is a sequence of 3 amino acids includes, for example, a sequence in which the amino acid adjacent to R² is Ala and a sequence in which the second and third amino acids (as read from left to right) is Lys-Ala, Arg-Ala and 30 Ala-Ala.

A value for R¹ of special interest when it is a sequence of three amino acids includes, for example, Ala-Lys-Ala, Ala-Arg-Ala and Arg-Ala-Ala, especially the latter.

A value for R^3 of particular interest when it is a sequence of three amino acids includes, for example, a sequence in which the first amino acid (as read from left to right, i.e. adjacent to R^2) is Ala.

A preferred value for R^3 when it is a sequence of three amino acids includes, for example, Ala-Ala-Ala and Ala-Arg-Ala.

A preferred value for R^3 when it is a single amino acid includes, for example, Ala, Gly and Azgly, especially Ala.

Particular values for R_a when it is alkyl include, for example, methyl, ethyl and propyl.

A preferred value for R_a includes, for example, hydrogen and methyl, especially hydrogen.

A suitable value for the hydrophobic residue P (which it will be appreciated is attached to the amino group of the N-terminal amino acid of R^1) includes, for example, an organic hydrophobic group such as a hydrophobic aliphatic, aromatic, heteroaromatic or mixed aliphatic/aromatic or aliphatic/heteroaromatic organic group of from 5 to 20 carbon atoms (and 1, 2 or 3 heteroatoms selected from oxygen, sulphur and nitrogen for heteroaryl - containing groups), for example a group of the formula $R-$, $R.CO-$, $R.SO_2-$, $R.O.CO-$, $R.NHCO-$, $R.O.CS-$, $R.S.CO-$, $R.NHCS-$, $R.S.CS-$ and $R.CS-$, in which R includes, for example, (5-10C)alkyl, aryl, heteroaryl, aryl(2-10C)alkyl, heteroaryl(2-10C)alkyl, diaryl(2-8C)alkyl, aryl(2-10C)alkenyl, arylcyclopropyl, (5-10C)cycloalkyl, (5-10C)cycloalkyl(2-6C)alkyl, 3-biphenyl, 4-biphenyl, 4-cyclohexylphenyl, 2-naphthyloxymethyl, 3-naphthyloxymethyl, phenoxyphenyl and tetrahydronaphthyl, an aryl or heteroaryl group of which values of R may bear one or more (1-4C)alkyl, halogeno, cyano or (1-4C)alkoxy substituents. One particular embodiment of the invention includes, for example, compounds of the formula I in which P is $R.CO-$ as defined above. A further particular embodiment of the invention includes for example, peptide compounds of the formula I wherein P is a hydrophobic aliphatic, aromatic or aliphatic/aromatic organic group of from 5 to 20 carbon atoms.

Particular values for R include, for example, when it is (5-10C)alkyl: pentyl, isopentyl, tert-pentyl, 2-methylpentyl, hexyl, isohexyl, 5-methylhexyl and octyl; when it is aryl: phenyl, naphthyl and indenyl; when it is heteroaryl: 2-, 3-, 5- or 6-indolyl, 2-, 3-, 5- or 6-indolinyl, 2-,

3-, 5- or 6- benzo[b]thiophenyl, thienyl, 2-, 4- or 5-benzothiazolyl, 2-, 4- or 5-benzoxazolyl, 2-, 4- or 5-benzimidazolyl, 1,4-benzodioxanyl attached at the 2-, 3-, 6- or 7-position and 2-, 3-, 5- or 6-benzofuranyl; when it is aryl(2-10C)alkyl: aryl(2-6C)alkyl (where the aryl portion includes, for example, any of the specific values for aryl given above and the (2-6C)alkyl portion includes, for example, methylene, ethylene, trimethylene, tetramethylene and pentamethylene) such as in 2-phenylethyl, 3-phenylpropyl, 4-phenylbutyl and 5-phenylpentyl; when it is heteroaryl(2-10C)alkyl: heteroaryl(2-6C)alkyl (where the heteroaryl portion includes, for example, any of the specific values for heteroaryl given above and the (2-6C)alkyl portion includes, for example, methylene, ethylene, trimethylene, tetramethylene and pentamethylene) such as in 2-(2-cyanobenzo[b]thiophen-5-yl)ethyl; when it is diaryl(2-8C)alkyl: diaryl(2-6C)alkyl such as 2,2-diphenylethyl, 3,3-diphenylpropyl and 4,4-diphenylbutyl; when it is aryl(2-10C)alkenyl: aryl(2-6C)alkenyl such as styryl, 3-phenylpropen-2-yl and 4-phenylbuten-1-yl; when it is arylcyclopropyl: phenylcyclopropyl, 1-naphthylcyclopropyl and 2-naphthylcyclopropyl; when it is (5-10C)cycloalkyl: cyclopentyl, cyclohexyl and 1-adamantyl; and when it is (5-10C)cycloalkyl(2-6C)alkyl: 2-(cyclohexyl)ethyl, 3-(cyclohexyl)propyl and 4-(cyclohexyl)butyl. A particular value for a substituent on an aryl group of R includes, for example, methyl, ethyl, chloro, bromo, iodo, methoxy, ethoxy and cyano.

The hydrophobic residue P also includes, for example, a hydrophobic L-amino acid, such as phenylalanine (Phe) and hydrogenated analogues thereof such as cyclohexylalanine (Cha), para-chloroPhe, 3-(2-thienyl)alanine, tyrosine (Tyr), Tyr(OMethyl), tryptophan (Trp), biphenylalanine, 3-(1-naphthyl)alanine, 3-(2-naphthyl)alanine and hydrogenated analogues thereof, 3-(1-adamantyl)alanine (Ada), Glu(OBenzyl), 3-(benzyloxy)Ala, 3-(benzylsulfanyl)Ala and 9-fluorenylGly, each of which may optionally bear on the N-terminus a hydrophobic aliphatic, aromatic, heteroaromatic or mixed aliphatic/aromatic or aliphatic/heteroaromatic organic group as defined and exemplified above. Alternatively, the hydrophobic amino acid may optionally bear, for example, a further sequence of 1 to 3 amino acids selected from any of the values for R¹ and R³ defined above. For example P includes the particular sequences Ala-Cha, Ala-Ala-Cha, Tyr-Ala-Ala-Cha, Tyr-Ala-Ala-Phe, Ala-Phe-Phe-Phe and Ala-Ala-Ala-Phe. The first amino acid of such further sequence of 1 to 3 amino acids (as read from left to right) may be an L- or D-amino acid and may also optionally bear a

hydrophobic aliphatic, aromatic, heteroaromatic or mixed aliphatic/aromatic or aliphatic/heteroaromatic organic group as defined or exemplified above.

Further particular values for P include, for example, 3-(benzyloxycarbonyl)propionyl-Phe, 3-(benzyloxycarbonyl)propionyl-Cha, 4-(benzyloxycarbonyl)butyryl-Phe, 4-(benzyloxycarbonyl)butyryl-Cha, (5-oxo-pyrrolidin-2-yl)carbonyl-Phe-Tyr, (5-oxo-pyrrolidin-2-yl)carbonyl-Glu(OBenzyl)-Tyr, acetyl-Glu(OBenzyl)-Tyr, diphenylmethyl.CONH.CH₂CH₂.CO-Cha, diphenylmethyl.CONH.CH₂CH₂.CO-Tyr, diphenylmethyl.CONH.CH₂CH₂CH₂.CO-Cha, diphenylmethyl.CONH.CH₂CH₂CH₂.CO-Tyr, diphenylmethyl.NHCO.CH₂CH₂CH₂.CO-Cha, diphenylmethyl.NHCO.CH₂CH₂CH₂.CO-Tyr, benzyl.NHCO.CH₂CH₂.CO-Cha, benzyl.NHCO.CH₂CH₂.CO-Tyr, N-acetyl-4-chloro-beta-hydroxyPhe, 4-phenoxyphenyl.NHCO-, benzyl.NHCO.CH₂CH₂.CO.(N-methylPhe), benzyl.NHCO.CH₂CH₂.CONH.CH(CHPh₂).CO, benzyl.NHCO.CH₂CH₂.CO-Tyr, 3,3-diphenylpropionyl, trans-cinnamoyl, 5-phenylvaleryl and 3-(2-cyanobenzo[b]thiophen-5-yl)propionyl.

A value for P of particular interest includes, for example, Ph.(CH₂)₄.CO-(5-phenylvaleryl (Phv)), Ph.(CH₂)₄.CS- and 3-(2-cyanobenzo[b]thiophen-5-yl)propionyl.

A preferred value for the hydrophobic residue P includes, for example, 3-(2-cyanobenzo[b]thiophen-5-yl)propionyl and 5-phenylvaleryl (Phv), especially the latter.

When R_c is a group of the formula -A¹-G¹, a particular value for A¹ when it is alkylene includes, for example, methylene, ethylene, propylene and butylene; a particular value for B² when it is (1-4C)alkylene includes, for example, methylene, ethylene and propylene; and a particular value for C³ when it is (1-3C)alkylene includes, for example, methylene, ethylene and propylene.

A particular value for -A¹-G¹ includes, for example, 3-guanidinopropyl and 4-(2-guanidinoethyl)phenyl.

A particular value for R⁴ when it is a sequence of 1 to 6 amino acids includes, for example, a sequence of L- amino acids independently selected from any of the values for R¹ and R³ defined above (such as Ala-Thr-Gly-OH), or their D- analogues, or a sequence containing both D- and L- amino acids, or an amide thereof, such as an amide derived from ammonia, an (1-4C)alkylamine (such as methylamine) or a di(1-4C)alkylamine (such as dimethylamine). A particular group of values for R⁴ includes, for example, those values where R⁴ is not a sequence of 1 to 6 amino acids.

A preferred value for R⁴ includes, for example, 4-carbamoyl-1-piperidyl (the residue of piperidine-4-carboxamide (Pip-NH₂)), 4-carboxy-1-piperidyl (the residue of piperidine-4-carboxylic acid (Pip-OH)), 4-(carbamoylmethyl)anilino (the residue of 4-aminophenylacetamide (Papa-NH₂)), 4-(carboxymethyl)anilino (the residue of 4-aminophenylacetic acid (Papa-OH)) and 4-(2-guanidinoethyl)anilino (the residue of 2-(4-aminophenyl)ethylguanidine (Pape-NHC(=NH)NH₂)). Pip-NH₂ and Papa-NH₂ are particularly preferred values for R⁴, and especially Papa-NH₂.

A particular group of values for R⁴ includes, for example, Pip-NH₂, Papa-NH₂, Pape-NHC(=NH)NH₂ and NHRc in which Rc is 3-guanidinopropyl, 2-morpholinoethyl or 4-(2-(2-hydroxyethoxy)ethyl-1-piperazinyl).

A preferred value for R² includes, for example, a group of the formula IIa and especially IIb, in which the ring carbon bearing the -NH- group has the S configuration.

A particular group of chemical compounds of formula I includes, for example, chemical compounds in which R¹ is a sequence of five L-amino acids and R³ is a single L-amino acid, which sequence R¹ and R³ have any of the values defined above, including the particular and preferred values for R¹ and R³. Within this group, a sub-group of particularly preferred peptides derivatives includes, for example, those in which R² is a group of the formula IIa and especially IIb. A further sub-group of particularly preferred chemical compounds includes, for example, those in which R⁴ is -Pip-OH, -Pip-NH₂, -Papa-OH or Papa-NH₂. An especially preferred sub-group of compounds includes, for example, those in which R³ taken together with R⁴ is Ala-Pip.NH₂ or Ala-Papa-NH₂.

A preferred group of chemical compounds of the invention include, for example, those in which R¹ is a sequence of 5 L-amino acids represented as AA1-AA2-AA3-AA4-AA5 in which:

AA1 is selected from Ala, Ile, Tyr, Val, Glu, Lys, Arg, Gly, Gap, GapMe₄ and 3,3,3-trifluoroalanine, particularly Ala, Ile, Arg, Gap and GapMe₄, especially Ala, Arg and GapMe₄, and more especially Ala and Arg;

AA2 is selected from Ala, Lys, Glu, Sar, Val, Arg, Gly, Pro, Ile, Tic, 3,3,3-trifluoroalanine and N⁶-diethylLys, particularly Ala, Arg, Ile, Lys and Tic, especially Ala, Arg, Lys and Ile and more especially Ala and Arg;

AA3 is selected from Ala, His, Gln, Val, Thr, Glu, Gly, Asp, Asn and N³-diethylDap, particularly Ala, His, Asp and Asn, especially Ala and Asn and more especially Ala;

AA4 is selected from Ala, Lys, Asn, Arg, Thr, Glu, Sar, Gly, Pro, His and N⁶-diethylLys, particularly Ala, Arg, Lys and His, especially Ala, Arg and His and more especially Ala; and AA5 is selected from Thr, Val, Ala, Gly, Dap, Dab, Pro, Hyp, Asn and N³-diethylDap (especially Thr and Val); and

- 5 R³ is a single amino acid selected from Ala, Gly, Dap, azaalanine and azaglycine (especially Ala, Gly and azaglycine);
- and wherein P, R² and R⁴ have any of the values, including the particular and preferred values, defined above. Within this group, a particular sub-group of compounds include, for example, those in which the sequence AA1-AA2-AA3-AA3-AA5 is selected from Ala-Ala-Ala-Lys-
- 10 Val, Ile-Ala-Ala-Arg-Thr, Arg-Ala-Ala-Ala-Val, Arg-Ala-Ala-Ala-Thr, Ala-Ala-Ala-Arg-Val, Ala-Arg-Ala-Arg-Val, Ala-Ile-Ala-Arg-Val, Ala-Arg-Ala-His-Val, Ala-Ala-Asn-Arg-Val, Ala-Arg-Ala-Ala-Thr, Ala-Arg-Ala-Arg-Thr, Gap-Ala-Ala-Ala-Thr, GapMe₄-Ala-Ala-Ala-Thr and Ala-Ala-Ala-Arg-Thr. Compounds in which R⁴ is Pip.NH₂, Papa.NH₂ and Pape.NHC(=NH)NH₂ are preferred.

- 15 A further preferred group of chemical compounds of the invention include, for example, those in which R¹ is a sequence of 3 L-amino acids represented as AA1-AA2-AA3 in which:

AA1 is selected from Ala, Ile, Tyr, Val, Glu, Lys, Arg, Gly, Gap, GapMe₄ and 3,3,3-trifluoroalanine, particularly Ala, Ile, Arg, Gap and GapMe₄, especially Ala, Arg and GapMe₄,

- 20 and more especially Ala and Arg;

AA2 is selected from Ala, Lys, Glu, Sar, Val, Arg, Gly, Pro, Ile, Tic, 3,3,3-trifluoroalanine and N⁶-diethylLys, particularly Ala, Arg, Ile, Lys and Tic, especially Ala, Arg, Lys and Ile and more especially Ala and Arg;

AA3 is selected from Ala, His, Gln, Val, Thr, Glu, Gly, Asp, Asn and N³-diethylDap,

- 25 particularly Ala, His, Asp and Asn, especially Ala and Asn and more especially Ala;; and

R³ is a sequence of 3 L-amino acids represented as AA6-AA7-AA8 in which:

AA6 is selected from Gly, Leu, Lys, Ala, Pro, Glu, Sar, His and Dap (especially Ala);

AA7 is selected from Pro, Ala, Lys, Arg, Glu, Sar, Gly, Oic and Dic (especially Ala and Arg); and

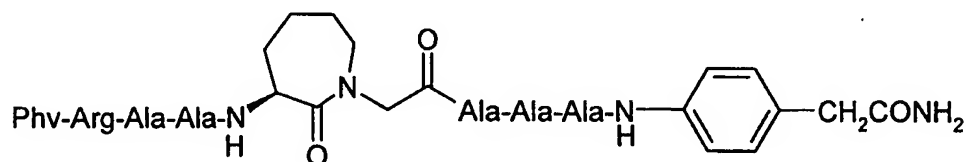
- 30 AA8 is selected from from Ala, Gly, Dap, azaalanine and azaglycine (especially Ala, Gly and azaglycine); and P, R² and R⁴ have any of the values, including the particular and preferred values, defined above. Within this group, a particular sub-group of compounds includes, for

example, those compounds in which the sequence AA1-AA2-AA3 is selected from and the sequence AA6-AA7-AA8 is selected from Ala-Ala-Ala and Ala-Arg-Ala. Compounds in which R⁴ is Pip.NH₂, PAPA.NH₂ and Pape.NHC(=NH)NH₂ are preferred.

5 A further preferred aspect of the present invention comprises chemical compounds of the formula I which contain an arginine residue, particularly compounds in which the first amino acid residue of R¹ (as read from left to right) when it is a sequence of 5 L-amino acids is arginine (such as Arg-Ala-Ala-Ala-Ala-Val and Arg-Ala-Ala-Ala-Ala-Thr) and compounds in which the first amino acid residue of R¹ when it is a sequence of 3 L-amino acids is arginine (such as when R¹ is Arg-Ala-Ala and R² is Ala-Ala-Ala.)

10 Compounds of the invention which are of particular interest include, for example, the specific embodiments set out hereinafter in the accompanying Examples, or a pharmaceutically acceptable salt thereof, which are provided as further features of the invention.

15 (SEQ ID NO:1)



and with organic acids such as acetic, oxalic, tartaric, mandelic, p-toluenesulphonic, methanesulphonic, trifluoroacetic acids and the like, and for chemical compounds that are sufficiently acidic, for example those having a free carboxylic acid group, salts with bases forming physiologically acceptable cations, such as salts with alkali metal (such as sodium and potassium), alkaline earth metal (such as magnesium and calcium), aluminium and ammonium salts, as well as salts with suitable organic bases such as ethanolamine, methylamine, diethylamine, isopropylamine, trimethylamine and the like.

As stated above, the chemical compounds of formula I, or a pharmaceutically acceptable salt thereof, will have beneficial pharmacological effect in warm-blooded animals (including man) in a range of autoimmune diseases or medical conditions, to treat symptoms or as a disease modifying agent or as a prophylactic treatment. Such diseases may include, for example, rheumatoid arthritis, multiple sclerosis, Goodpasture's syndrome, idiopathic thrombocytopenic purpura, juvenile rheumatoid arthritis, coeliac disease, systemic lupus erythematosus, ankylosing spondylitis, Sjogren syndrome, myasthenia gravis, Type 1 (insulin dependent) diabetes, Hashimoto's disease, Grave's disease, Addison's disease, scleroderma, polymyositis, dermatomyositis, pemphigus vulgaris, bullous pemphigoid, autoimmune haemolytic anaemia, pernicious anaemia, glomerulonephritis, graft rejections and such like, especially rheumatoid arthritis and multiple sclerosis.

The utility of the chemical compounds of the formula I, or an amide thereof, or a pharmaceutically acceptable salt thereof, may be assessed using a variety of standard tests and clinical studies, including those described in International Patent Application, Publication Nos. WO92/02543, WO93/05011 and WO95/07707 (or modifications thereof) and those described below. The chemical compounds of formula I show significant activity in one or more of such tests or studies.

Test A: Purified HLA-DR peptide in vitro binding assay. (This assay may be used to demonstrate the binding of the peptide compounds of formula I to disease-associated MHC class II molecules.) 30µl of biotin-FHA₃₀₇₋₃₂₀ (FHA (307-320) peptide, derivatised with long-chain biotin at the N-terminus, Biotin-Ahx-Pro-Lys-Tyr-Val-Lys-Gln-Asn-Thr-Leu-Lys-Leu-Ala-Thr-Gly-OH) at 800nM in phosphate buffered saline solution (PBS) is incubated with 30µL of purified HLA-DR4Dw4 at a concentration between 0.5 and 5 µg/ml in V-welled micro-titre plates (Nunc) for 48 hours with or without inhibitor peptides. At the end of the incubation period 100µl of the incubate is transferred to Enzyme Linked ImmunoSorbant

Assay (ELISA) plates (Nunc) previously coated with an anti-MHC antibody (L243 - American Type Culture Collection (ATCC) HB55 as described in Lampson and Levy (1980) J. Immunol. 125, 293-299) at a concentration of 10 µg/ml for 1 hour at room temperature and blocked thereafter for 1 hour with 1% bovine serum albumin (BSA) in PBS and 0.05% Tween

5 20. After a further 1 hour period the unbound peptide is washed away and a 1/4,000 dilution of streptavidin peroxidase (Sigma) in PBS with 0.01% of a suitable detergent such as NP-40 (Sigma) added for 2 hours at room temperature. After further washing tetramethylbenzidine (TMB) substrate solution (1 TMB tablet (Sigma) in 10 mls of 0.1M citrate/acetate buffer, pH 6.0 with 36 µl urea hydrogen peroxide (UHPO) (Fluka)) is added to each of the plates. The

10 reaction is stopped by adding 2M sulphuric acid (10µl per well) and the absorbance read at 450nm to quantify the amount of peptide bound. The inhibitory activity of peptides is obtained by plotting absorbance against concentration.

The purified HLA-DR4Dw4 may be obtained as follows:-

- (i) Expression of HLA-DR in the Baculovirus system
- 15 The expression of recombinant proteins in insect-cells from baculovirus vectors is an established procedure to obtain high yields of recombinant protein [Luckow, VA & Summers, MD (1988) Biotechnology 6 47-551]. To enable the expression of the heterodimeric HLA-DR eg. HLA-DR4Dw4 from a single recombinant baculovirus vector (as opposed to having separate recombinant viruses for the α and β chains and then doing a co-infection), a double-
- 20 recombinant baculovirus is constructed which carries both the α and β chains.

A cDNA encoding the sequence of the α polypeptide is cloned into the transfer vector pacYM1 [Matsuura, Y; Possee, RD; Overton, HA & Bishop, DHL (1987 J. Gen. Virol 68, 1233-1250] to place expression of the protein under the control of the polyhedrin promoter. The unit is inserted into the baculovirus genome by homologous recombination in Sf21

25 insect-cells to create a single recombinant baculovirus for the α chain. The techniques for the culture and infection of insect-cells, for the homologous recombination and detection/isolation of recombinant viruses are all fully described by Summers, MDD & Smith GE (1987) [A Manual of Methods for Baculovirus Vectors and Insect-cell Culture Procedures; Texas Agricultural Experiment Station, Bulletin No. 1555]. The molecular

30 genetic techniques used to construct the recombinant vectors are likewise readily available in the literature and are most fully described by Sambrook, J; Fritsch, EF & Maniatis T, (1989)

[Molecular Cloning. A Laboratory Manual. 2nd Edition. Cold Spring Harbor Laboratory Press].

To create the double-recombinant baculovirus, a cDNA encoding the β chain is cloned into the transfer vector pAcUW1 [Weyer, U; Knight, S & Possee, RD (1990) J. Gen. Virol., 71, 1525-1534] to place expression of the protein under the control of the P10 promoter. The unit is then inserted into the genome of the single recombinant baculovirus carrying the α chain. Double-recombinant viruses are detected by spotting insect-cells, infected with randomly picked viruses from the transfection, onto membranes and reacting them with a monoclonal antibody e.g. L243 which specifically recognises the HLA-DR heterodimer. Binding of the antibody to Sf21 insect-cells is detected using standard flow cytometry techniques, readily available in the literature. Stable, double-recombinant baculovirus expressing HLA-DR are plaque-purified.

(ii) Purification of HLA-DR from insect-cells

The method used is a modification of that described by Gorga et al 1987. (Gorga et al 1987. J. Biol. Chem. 262, 16087-16094). HLA-DR expressing baculovirus/Sf21 cells (10L which is approximately equal to 2×10^{10} cells) are solubilised in 100ml of 5mM EDTA (sodium salt), 50mM Tris-HCL, pH 8.5, 2% NP40, 150mM NaCl, 1mM iodoacetamide, 1mM PMSF by homogenisation with 10 strokes of a teflon glass homogeniser. The homogenate is spun at 100,000g for 1 hour and the supernatant collected. The anti-HLA-DR monoclonal antibody LB3.1 (Gorga et al 1986, Cell. Immunol. 103, 160-172) covalently coupled at a ratio of 50mg of L243 to 10ml of Protein A-Sepharose fast flow (Pharmacia) and pre-incubated with 10mM Tris-HCl, pH 8.0, 0.1% NP-40 is incubated overnight with the supernatant. The resin is then put into a column and washed with 10mM Tris-HCl, pH 8.0, 0.1% NP-40 (20 column volumes) followed by 0.15 M NaCl, 50mM Na_2HPO_4 , pH 7.0, 1% octylglucoside (20 column volumes). The HLA-DR is eluted with 50mM diethylamine pH 11.0, 0.15 M NaCl, 1% octylglucoside. Column fractions are immediately neutralised with 1 M Tris-HCl pH 8.0 and concentrated by ultracentrifugation through a centricon-10 membrane. Protein content is determined by a BCA protein assay (Pierce) and purity by SDA-PAGE electrophoresis.

In general, the chemical compounds of formula I as defined above which were tested in test A showed significant inhibition at a concentration of about $10\mu\text{M}$ or much less.

A further preferred aspect of the present invention comprises a peptide derivative of the formula I, or a pharmaceutically acceptable salt thereof, which does not bind to HLA-DR3

but binds to HLA-DR1 and/or HLA-DR4Dw4 and/or HLA-DR4Dw14. HLA-DR3 is a common HLA-DR allele which is not associated with rheumatoid arthritis. Accordingly, in rheumatoid arthritis patients who carry HLA-DR3 as one of their alleles (which is approximately one third of the total rheumatoid arthritis patients), such a peptide derivative of the formula I will not interfere with the normal role of HLA-DR3 in the host-defense function. The use of such a peptide derivative is therefore particularly advantageous for treating rheumatoid arthritis patients as it will result in less immunosuppression than would occur with a non-selective DR binder.

As a variant to test A, the ability of a peptide of the invention to bind to one or more HLA-DR molecules was assessed as follows:

(i) Purification of HLA-DR Types from Cell lines

The method used was a modification of that described by Gorga et al, 1987, J.Biol.Chem. 262, 16087-16094. Human HLA-DR antigens were purified from various cell lines by immunoaffinity chromatography. Briefly, 1×10^9 - 5×10^9 pelleted cells of the appropriate cell line selected from Hom 2 (source of DR1), BBF (source of DR2), AVL-B (source of DR3), JAH (source of DR4Dw4), JHAF (source of DR4Dw13) or PE117 (source of DR4Dw14) were solubilised at approximately 4°C in 50 ml of 5 mM EDTA (sodium salt), 50mM Tris-HCL pH 7.4, 2% NP40, 150 mM NaCl, 1 mM iodoacetamide, 1 mM PMSF, by homogenisation with 10 strokes of a teflon glass homogeniser. The homogenate was spun at 100,000 g for 1 hour and the supernatant collected. The anti-HLA-DR monoclonal antibody LB3.1 (Gorga et al, 1986, Cell.Immunol., 103, 160-173) covalently coupled to CNBr-Sephrose 4B (Pharmacia) was pre-equilibrated with 150 mM NaCl, 50 mM Tris-HCL, pH 7.4, 0.1% NP-40 and incubated overnight with the supernatant. The resin was then packed in a column and washed with 0.15 M NaCl, 50 mM Tris-HCL, pH 7.4, 1% octylglucoside (20 column volumes). The HLA-DR was eluted with 50 mM diethylamine pH 11.0, 0.15 M NaCl, 1% octylglucoside. Column fractions were immediately neutralised with 0.5 M HEPES NaOH pH 7.4. Protein content was determined by a Biorad protein assay and purity by SDS-PAGE electrophoresis.

(ii) Peptide Selectivity Binding Assays

200 nM biotin-FHA₃₀₇₋₃₂₀ in phosphate buffered saline (PBS) was incubated with either purified HLA-DR1, DR2, DR4Dw4, DR4Dw13 or DR4Dw14 (2-20µg/ml) in V-well microtitre plates (Nunc) with or without inhibitor peptides in assay buffer (PBS, 0.01% NP40

(Sigma.)) For DR3 inhibition, 400 nM Biotin-Ahx-(D)Ala-Ala-Ala-Che-Ile-Ala-Ala-Ala-Thr-Leu-Lys-Ala-Ala-(D)Ala-OH was incubated with purified DR3 (20µg/ml), and incubated as above. After 48 hours, the incubates were treated, and absorbance readings taken as described in Test A. The inhibitory activity of peptides, expressed as IC₅₀ values, was

5 calculating using Microcal Origin software on a PC.

Test B: Inhibition of T-cell activation in vitro. (This assay may be used to demonstrate the ability of the peptide compounds of formula I to inhibit a T-cell immune response mediated by or through an MHC class II molecule).

Inhibitor peptides were tested for the ability to block stimulation of the B52.24 murine
10 T-cell hybridoma line which responds to the FHA₃₀₇₋₃₂₀ peptide (H-Pro-Lys-Tyr-Val-Lys-Gln-Asn-Thr-Leu-Lys-Leu-Ala-Thr-Gly-OH) presented by HLA-DR4Dw4 molecules. B52.24 was produced by the fusion of lymph node T-cells taken from FHA₃₀₇₋₃₂₀ immunised HLA-DR4Dw4 transgenic mice (International Patent Application, Publication No WO95/03331) with the BW5147 murine T-cell lymphoma line (White et al (1989) J. Immunol. 143, 1822) as
15 outlined in Woods et al (1994) J. Exp. Med. 180, 173-181 and following the general methods for the generation of T-cell hybridomas given in Current Protocols in Immunology, Volume 2, 7.21.

Inhibitor peptides at concentrations between 100 and 0.1µM (or lower) were mixed with the antigenic peptide FHA₃₀₇₋₃₂₀ in either varying concentrations between 100 and 0.1 µM
20 or at a fixed concentration of 10 µM by dilution in RPMI-1640 culture media (Gibco) in a 96-well microtitre plate (Nunc) in a final volume of 100 µl. HLA-DR4Dw4 expressing B cells such as the JAH EBV transformed lymphoblastoid cell line (European Culture Collection ECACC 85102909) or B cells taken from an HLA-DR4Dw4 homozygous individual and transformed with Epstein Barr virus according to the method described in Current Protocols in
25 Immunology 7.22.1 were fixed using glutaraldehyde by suspension in 1% glutaraldehyde (Sigma) at 4 x 10⁶ cells/ml for 30 seconds, after which an equal volume of 200mM lysine (Sigma) was added for 3 minutes. The cells were recovered by centrifugation at 300g, washed in RPMI-1640 and added to the microtitre plates containing antigen and inhibitor compounds at a concentration of 2 x 10⁵ cells per well. The microtitre plates were incubated for 2 hours at
30 37°C and 5% CO₂.

The microtitre plates were then washed in RPMI-1640 by centrifugation at 300g and aspiration twice before the addition of the B52.24 T-cell hybridoma line at a concentration of

10⁵ cells per well in culture medium (RPMI-1640, 10% foetal calf serum (Gibco) and 2mM glutamine (Gibco)). The microtitre plates were then incubated for a further 2 days at 37°C and 5% CO₂. The plates were then centrifuged at 300g for 10 minutes and 150µl of supernatant removed from all wells to be frozen at -20°C prior to bioassay for IL-2 content.

5 The culture plates containing supernatants to be assayed were left at room temperature to thaw and 100µl of supernatant was transferred to fresh 96 round bottomed well plates. 1:1 serial dilutions of IL-2 were carried out using culture media (RPMI-1640 (Gibco), 10% foetal calf serum (Advanced Protein Products), 100 µg/ml streptomycin and 100 U/ml penicillin (Gibco), 2mM L-glutamine (Gibco) and 50 µM 2-mercaptoethanol (Sigma)), to produce a
10 standard curve of 250 units/ml to 0.04 units/ml IL-2 final. An IL-2 dependent T-cell line such as CTLL-2 cells (Nature (1977) 268 154-156) or HT-2 cells (J. Immunol. Methods (1987) 94-104) were harvested and washed twice using culture media prior to resuspension at 5 x 10⁴ cells/ml. 100µl of IL-2 dependent T-cell suspension was added to each well of the standard curve and test samples. The culture plates were incubated for 72hrs at 37°C and 5% CO₂.
15 After which, 20µl (1mCi) of 3H-Thymidine (Amersham International) was added to each well and the plates returned to the incubator for a further 16hrs. The contents of each plate were harvested onto glass fibre filter mats and the radioactivity measured using a betaplate scintillation counter.

 In general, the peptide compounds of formula I as defined above which were tested in
20 test B showed significant inhibition at a concentration of about 10µM or much less.

Test C: Peptide stimulated DTH (delayed type hypersensitivity) in BALB/C mice. (The assay may be used to demonstrate in vivo activity of peptide compounds of formula I in an animal model). Balb/c female mice (18-20g), 5 per group, were immunised sub-cutaneously on the flank with 0.1ml of an emulsion of ovalbumin (Sigma) (2mg/ml in saline) mixed 1:1
25 (v/v) with complete Freund's adjuvant (Sigma). Seven days later footpad thickness was determined using a dual caliper micrometer followed by a challenge in one hind footpad with a 30µl sub-plantar injection of 1% heat-aggregated ovalbumin protein in saline. Twenty-four hours after antigen challenge, footpads were measured and the DTH response calculated as the percentage increase in footpad thickness in the injected footpad compared to contralateral
30 control. Inhibitors were administered by 3-day osmotic mini-pumps (Alzet) implanted 24 hours prior to antigen challenge at doses ranging from 10mg/kg/day to 0.1 µg/kg/day. The degree of inhibition was calculated by subtracting the value for swelling of inhibitor treated

footpads from that of the vehicle dosed controls, dividing by the control value and multiplying by 100%.

In general, the peptide compounds of formula I as defined above which were tested in Test C showed significant inhibition at a dose of about 1mg/kg/day or much less, without any overt toxicological or other untoward pharmacological effect.

Test D: (This assay may be used to demonstrate in vivo activity of peptide compounds of formula I in an animal model of arthritis).

Balb/c female mice (19-21g, 5-10/group) are immunised on day 0 and boosted on day 7 with a sub-cutaneous injection of 0.1ml of an emulsion containing equal volumes of 2mg/ml methylated bovine serum albumin (met-BSA, Sigma) in saline and complete Freund's adjuvant (Sigma) supplemented with 2.5mg/ml Mycobacterium tuberculosis (MTB, strains C, DT and PN, MAFF, Weybridge, Surrey) thus giving a final MTB concentration of 3.5 mg/ml. An additional 0.1 ml i.p injection of 10^9 Bordetella pertussis organisms (Wellcome Pertussis vaccine) in saline is given at the same time. Fourteen days later, animals are challenged into one knee joint with a 10 μ l intra-articular injection containing 100ug of met-BSA in saline using a 30G needle and hamilton syringe. The contralateral knee is injected with a similar volume of saline and serves as a control. The degree of inflammation/swelling associated with both knees is determined 13 days later by measuring using a dual-caliper micrometer. This is achieved by making an incision with blunt-ended scissors and forceps into the skin approximately 5mm above and below the knee and continuing along the side of the knee to form a flap which is then carefully cut away to expose the underlying joint. Measurements are made across the widest part of the knee, in the horizontal plane, on the flexed limb held in a fixed position. Percentage increase in inflammation in the antigen-injected knee compared to control is calculated according to the formula: [antigen-injected knee thickness - saline-injected knee thickness/saline-injected knee thickness] x 100. Inhibitors are administered using 14 day osmotic mini-pumps (Alzet) implanted 24 hrs before antigen challenge at doses ranging from 10mg/kg/day to 0.1ug/kg/day. The percentage inhibition of inflammation/swelling is calculated from the thickness measurements by subtracting the value for swelling in the inhibitor-treated group from that of the vehicle dosed controls, dividing by the control value and multiplying by 100. Additional assessments of disease involve 1) the histological evaluation of inflammation, synovitis and cartilage/bone erosions carried out on

fixed knee sections stained with haemotoxylin and eosin and 2) the determination of levels of acute phase reactants in serum, serum amyloid P and/or haptoglobin.

Peptide compounds of formula I as defined above may show in Test D significant inhibition at a dose of about 10 mg/kg/day or much less.

5 By way of illustration of the pharmacological activity of particular peptide compounds of the formula I, the compound of Example 1 showed significant binding to HLA-DR4Dw4 in Test A (or a variant to test A described hereinbefore) at a concentration of 0.1 micromolar or much less, and was active at <0.1 mg/kg/day in Test C. The compound of Example 1 showed significant binding to HLA-DR1 and HLA-DR2 in addition to HLA-DR4Dw4, but no
10 significant binding ($IC_{50} > 100$ micromolar) to HLA-DR3. The compound showed good aqueous stability at pH3 and pH7.6 and, in the form of an extracted polymer depot formulation, showed minimal loss due to degradation on extrusion and minimal degradation on release from such a depot formulation.

A peptide compound of formula I may be prepared by any process well known in the
15 art of peptide chemistry to be applicable to the synthesis of analogous peptides.

A peptide compound of formula I may be obtained, for example, by procedures analogous to those disclosed in "Solid Phase Peptide Synthesis: A practical approach" by Atherton and Sheppard (published by IRL press at Oxford University Press, 1989). "Solid Phase Peptide Synthesis" by Stewart and Young (published by the Pierce Chemical Company,
20 Illinois, 1984), "Principles of Peptide Synthesis" (published by Springer-Verlag, Berlin, 1984), and a series of books "Amino Acids, Peptides and Proteins" (volumes 1 - 25; volume 25 published in 1994) (published by the Royal Society of Chemistry, Cambridge, UK).

Preferably, a peptide compound of formula I is prepared by solid phase sequential synthesis. Using this technique, the amino acid which is to become the C-terminus amino
25 acid of the peptide is protected at the alpha-amino group, and, if necessary, in the side chain and coupled to a solid support, for example a resin, such as 2-chlorotritylchloride resin or Merrifield resin (chloromethylpolystyrene-divinylbenzene) if a free carboxylic acid is required after cleavage, or Rink Amide resin (4-(2',4'-dimethoxyphenyl-Fmoc-aminomethyl)-phenoxy resin) or Rink Amide MBHA resin (N-(4-[2',4'-dimethoxyphenyl-Fmoc-aminomethyl)-phenoxyacetamido-norleucyl)-4-methyl benzhydrylamine resin (all available
30 from Calbiochem-Novabiochem) if a carboxamide is required after cleavage, whereafter the protecting group on the alpha-amino group is removed. The amino acid which is to be

attached to the C-terminus amino acid is protected at the alpha-amino group and, if necessary, in the side chain and coupled to the C-terminus amino acid which remains attached to the solid support. The stepwise process of deprotection of the alpha-amino group and coupling to the next amino acid is repeated to give a protected or unprotected polypeptide attached to the solid support. The group R² of formula II is incorporated into the sequence by using an appropriately protected 3-amino-2-oxoperhydroazepine-1-alkanoic acid, such as (3S)-3-(9-fluorenylmethoxycarbonylamino)-2-oxoperhydroazepine-1-acetic acid, obtained as described in the Examples (or by analogy therewith), in place of a protected amino acid. The protected or unprotected polypeptide is released from the solid support by standard procedures, for example using a mixture of trifluoroacetic acid, triethylsilane and water.

It will be appreciated that a side-chain protecting group may be cleaved under the conditions used to release the peptide from the solid support, or may be cleaved as a separate step prior or subsequent to release of the peptide from the solid support. It will also be appreciated that the procedure to build up the polypeptide may be modified by using a sequence of two or more suitable protected amino acids in a particular coupling step.

The synthesis may use manual techniques or be carried out automatically, employing for example, an Applied Biosystems 431A or 430A peptide synthesiser, an Advanced Chemtech ACT 357 peptide synthesiser or similar automatic peptide synthesiser, or a combination of both techniques may be used.

During the assembly of the peptides, the amino acid functional groups not taking part in the reaction are protected by various functional groups. For example, the N-terminal and side chain amino groups may be protected by using 9-fluorenylmethoxycarbonyl (Fmoc), t-butoxycarbonyl (Boc), biphenylisopropoxycarbonyl (Bpoc), 2-[3,5-dimethoxyphenyl]propyl-2-oxycarbonyl (Ddz), adamantyloxycarbonyl (Adoc), allyloxycarbonyl (Aloc), 2,2,2-trichloroethoxycarbonyl (Troc), benzyloxycarbonyl and various substituted benzyloxycarbonyl groups. These protecting groups can be cleaved when required by the standard techniques (e.g. acid or base treatment, catalytic hydrogenolysis and Pd(0) treatment or zinc/acetic acid treatment).

Suitable protecting groups used for the protection of the side chain guanidino group in the peptides containing an arginine residue include a nitro, adamantyloxycarbonyl, 4-methoxy-2,3,6-trimethylbenzenesulphonyl (Mtr), 2,2,5,7,8-pentamethylchroman-6-sulphonyl (Pmc) and (especially) 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulphonyl (Pbf) group.

Suitable protecting groups used for the protection of a side chain hydroxy group include t-butyl, benzyl and trityl (Trt). Suitable protecting groups for the side chain imidazole group in the peptides containing a histidine residue include a trityl, benzyl, tosyl, dinitrophenyl, Adoc, Boc or Fmoc group.

- 5 Suitable protecting groups used for the protection of a side chain carboxyl group include various esters (e.g. methyl, ethyl, t-butyl, benzyl, nitrobenzyl, allyl and 9-fluorenylmethyl).

10 The protecting group cleavage reactions can be performed at temperatures in the range of 4°C to 40°C (preferably at or about ambient temperature) and over a period of time in the range of 10 minutes to 24 hours.

- 15 Suitable coupling methods used for the coupling of the individual amino acids include the commonly used azide, symmetrical anhydride, mixed anhydride and various active esters and carbodiimides. In the case of various carbodiimides (e.g. dicyclohexyl- or diisopropyl-carbodiimides), a number of additives (e.g. 1-hydroxybenzotriazole (HOBT) and N-hydroxysuccinimide) may also be added. In addition, the amino acid couplings can also be achieved by using a number of other reagents, e.g. 1H-benzotriazole-1-yl-oxy-tris-pyrrolidinophosphonium hexafluorophosphate (PyBOP), (2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) and (2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (HBTU). The coupling reactions can be performed at
20 temperatures in the range of -20°C and 40°C and over a period of time in the range of 10 minutes to 24 hours. A suitable medium for carrying out the coupling reactions includes, for example, N,N-dimethylformamide (DMF). A particularly suitable method includes the use of HBTU, HOBT and diisopropylethylamine in DMF.

- 25 These and other methods of peptide synthesis are exemplified in the International Patent Applications referred to herein.

- 30 A hydrophobic residue P which is a group of the formula R-, R.CO-, R.SO₂-, R.O.CO-, R.NHCO-, R.O.CS-, R.S.CO-, R.NHCS-, R.S.CS- and R.CS- (or such a group present as a substituent on a terminal amino group of P where P is a hydrophobic amino acid or a hydrophobic amino acid bearing further amino acids) may be incorporated, for example, as a final step by alkylation, acylation or other standard functional group modification of a terminal amino group (for example prior to or subsequent to release of the peptide from the support). When C-terminus modifications are required (to obtain a particular value for R⁴),

they may be performed after the peptide is synthesised, using conventional functional group modification. Alternatively a particular value for R^4 may be obtained by appropriate choice of the initial starting resin and/or the protected entity first coupled to the resin (for example by using a suitably protected group of the formula $H-R^4$). Typical examples of the preparation
5 of peptide compounds of formula I are provided in the examples hereinafter.

A typical procedure for measuring the stability of a peptide compound of the present invention is as follows, in which, to minimize microbial contamination and degradation, all equipment that is used to prepare peptide solutions is sterilized in an autoclave and all material transfers carried out in a Class II laminar flow cabinet. Approximately 20ml of
10 McIlvaine's citric acid-phosphate buffer solution at pH 3 or 7.6 containing 0.02% sodium azide is filtered into a 50ml bottle using a sterile 0.22 μ m filter unit and a 20ml syringe. Approximately 1.2 mg of peptide is accurately weighed in a capped vial. Using a sterile pipette tip, sufficient buffer solution is added to the peptide in the vial to give a peptide concentration of 0.1 mg/ml. The vial is capped and shaken to dissolve the peptide. Using a
15 sterile pipette tip, aliquots of approximately 1ml of the peptide solution are transferred to 10 HPLC vials, which are then capped. 5 vials are stored at -18 and 37°C. The area of the peptide peak for the solution is determined by HPLC using appropriate standards initially and after storage at -18 and 37°C for 1, 2, 3 and 4 weeks, using a fresh vial at each time point with duplicate sample injections. The percentage of peptide remaining after storage at 37°C at each
20 time point is determined from the ratio of the area of the peptide peak at each time point to the initial area. Preferred peptides of the present invention have greater than 90%, and preferably greater than 95%, of peptide remaining after storage at 37°C at both pH 3 and 7.6.

The peptide compound of formula I will generally be administered for therapeutic or prophylactic purposes to warm-blooded animals (including man) requiring such treatment in
25 the form of a pharmaceutical composition, as is well known in the pharmaceutical art.

According to a further feature of the invention there is provided a pharmaceutical composition which comprises a peptide compound of the formula I, or a pharmaceutically acceptable salt thereof, in association with a pharmaceutically acceptable diluent or carrier.

The composition may be in a form suitable for oral use, for example a tablet, capsule,
30 aqueous or oily solution, suspension or emulsion; for nasal use, for example a snuff, nasal spray or nasal drops; for vaginal or rectal use, for example a suppository; for administration by inhalation, for example as a finely divided powder or a liquid aerosol; for sub-lingual or

buccal use, for example a tablet or capsule; or for parenteral use (including intravenous, subcutaneous, intramuscular, intravascular or infusion), for example a sterile aqueous or oily solution or suspension. The composition may be in a form suitable for topical administration such as for example creams, ointments and gels. Skin patches are also contemplated.

- 5 Formulation in general is described in Chapter 25.2 of Comprehensive Medicinal Chemistry, Volume 5, Editor Hansch et al, Pergamon Press 1990.

In general the above compositions may be prepared in a conventional manner using conventional excipients. However, in the case of a composition for oral administration, it may be convenient for the composition to include a coating to protect the polypeptide active
10 ingredient from the actions of enzymes in the stomach.

A preferred composition of the invention is one suitable for oral administration in unit dosage form for example a tablet or capsule which contains from 2.5 to 500mg, and preferably 10 to 100 mg, of polypeptide in each unit dose, or one suitable for parenteral administration which contains from 0.5 to 100mg of polypeptide per ml, and preferably 1 to 10mg of
15 polypeptide per ml of solution.

A parenteral composition is preferably a solution in isotonic saline or isotonic dextrose buffered if necessary to a pH of 5 to 9. Alternatively, the parenteral composition may be one designed for slow release in which case the amount of polypeptide per unit dose is in general greater than that required when a conventional injectable formulation is used. A preferred
20 slow release formulation is a continuous release formulation, for example a formulation of the type described in European Patent Specification No. 58481 or, for peptide compounds of formula I containing at least one basic group, a formulation as described in International Patent Application, Publication No. WO93/24150. Certain peptide compounds of the present invention possess solubility characteristics which make them particularly suitable for the
25 manufacture and processing of slow release parenteral formulations, particularly formulations containing biodegradable polyesters such as polylactides, and for providing slow release formulations with beneficial release profiles. Furthermore, peptide compounds of the present invention containing one or more basic groups, particularly arginine, can also form peptide-polymer salts with acid-ended polyesters, such as polylactides, and such peptides and peptide-
30 polymer salts constitute a further aspect of the present invention. Certain such salts possess solubility characteristics which make them particularly suitable for the manufacture and processing of slow release parenteral formulations, for example as described in WO93/24150,

and for providing slow release formulations with beneficial release profiles and storage stability characteristics. A preferred slow release parenteral formulation contains from 1 to 100 mg (such as 5 to 50 mg) of polypeptide per unit dose. A preferred slow release parenteral formulation is also one designed for slow release over a period of at least 5 days.

- 5 Preferred peptide compounds of the present invention include those which, when in the form of an extruded polymer depot formulation, show minimal loss due to degradation on extrusion or which show minimal degradation on release from such a depot formulation. Typical procedures for measuring the level of degradation of a peptide compound of the present invention are as follows:-

10 Preparation of extruded polymer depot formulation of peptide

- About 20 mg of peptide is accurately weighed and sufficient polymer (50/50% molar poly(D,L-lactic acid/glycolic acid) copolymer of approximate weight average molecular weight 20kD and approximate polydispersity of 1.7 as determined by size exclusion chromatography relative to polystyrene standards) added to produce an approximate 20% w/w mixture. This is dissolved in anhydride-free glacial acetic acid to produce an approximate 15 10% w/v solution. The solution is freeze dried and the resulting freeze dried product is stored under vacuum prior to use.

- About 100mg of freeze dried material is loaded into the barrel of a small laboratory extruder and the plunger pushed down to consolidate the sample. The extruder is heated to 20 between 90 and 95 °C and held at this temperature for 10 minutes before the freeze dried material is extruded under pressure to give a cylindrical extrudate of approximately 1mm in diameter.

Analysis of peptide content of extruded polymer depot formulation of peptide

- Two approximate 5mm lengths of extruded polymer depot containing peptide are 25 accurately weighed and each dissolved in 1ml of anhydride-free glacial acetic acid in separate 25ml volumetric flasks. After about 1.5 hours the volume of each is made up to 25ml with distilled water, causing the polymer to precipitate. The solids are filtered off using a 0.5µm Millex PTFE filter and the solutions, A, collected.

- A series of standard solutions are prepared from a stock solution of peptide in distilled 30 water at 0.5mg/ml and a stock solution of polymer in anhydride-free glacial acetic acid at 2.5mg/ml as follows with each solutions made up to 10ml with distilled water:

Concentration of peptide($\mu\text{g/ml}$)	Volume of stock polymer solution (μl)	Volume of stock peptide solution (μl)
50	1000	1000
40	1000	800
30	1000	600
20	1000	400
10	1000	200
5	1000	100
0	1000	0

Each standard is filtered through a 5 μm Millex PTFE filter and an aliquot of filtrate, together with aliquots of the solutions A, analysed by HPLC using duplicate sample injections. The peptide content of the extruded polymer depot formulation of peptide is calculated from the concentration of peptide in solutions A, which is determined by comparing the area of the peptide peak in solutions A with the area of the peptide peak from the standard solutions. Preferred peptides of the present invention show minimal loss due to degradation on extrusion and thus the peptide content of the extruded polymer depot formulation is close to the approximate theoretical value of 20% w/w.

Degradation of peptide on *in vitro* release from an extruded polymer depot

A solution of McIlvaine's citric acid-phosphate buffer solution at pH 7.6 containing 0.02% sodium azide, is filtered through a 0.22 μ filter and stored at 4°C. Approximately 10 mg of extruded polymer depot containing peptide is placed in two small vials and 2ml of the buffer solution added. The vials are then capped and stored in a water bath at 37°C for a month. At suitable time points over a month, three 0.6ml aliquots of release medium are removed from each vial and either analysed by HPLC or stored frozen in an HPLC vial at -18°C prior to analysis by HPLC. 1.8ml of the buffer solution is added to each vial containing the depot to replace the release medium that has been removed at each time point.

The average amount of intact peptide in the release medium at each time point is determined by HPLC using duplicate sample injections by comparing the area of the peptide peak in the release media with the area of the peptide peak from standard buffer solutions of

peptide at known concentrations. The approximate average amount of peptide degradation products in the release media at each time point is determined by HPLC by comparing the area of additional new peaks in the release media with the area of the peptide peak from standard buffer solutions of peptide at known concentrations and assuming the extinction coefficient has not changed. The average cumulative *in vitro* release profile of intact peptide and total peptide (intact peptide and peptide degradation products) is determined from the amounts of intact peptide and peptide degradation products in the release medium at each time point.

Preferred peptides of the present invention show minimal degradation on *in vitro* release and thus show total peptide degradation products of less than 10% and preferably less than 5% of total peptide after a month of *in vitro* release into McIlvaine's buffer solution at pH 7.6 at 37°C.

The composition of the invention will generally be administered to man such that, for example, a daily dose will be from 10 micrograms to 5000mg, preferably 0.1 to 100mg, for a 70kg patient, given in divided doses as necessary. The precise amount of composition administered and the route and form of administration may depend on the size, age and sex of the person being treated and on the particular disease or medical condition being treated and its severity, according to principles well known in the medical art.

A peptide compound of formula I, or a pharmaceutically acceptable salt thereof, may also be advantageously administered for therapeutic or prophylactic purposes together with one or more other pharmacological agents known in the general art to be of value in treating or relieving the symptoms of (or to be a disease modifying agent of) one or more of the diseases or medical conditions referred to hereinabove, such as a NSAID (such as ibuprofen or piroxicam), an analgesic (such as paracetamol), a corticosteroid, a muscle relaxant, a lipoxigenase inhibitor, methotrexate, azathioprine, D-penicillamine, Cyclosporin A or a monoclonal antibody therapy (such as anti-CD4 or anti-TNF). In diabetes the peptide compound may be co-administered with insulin or other therapies for diabetes or diabetes complications, (such as an aldose reductase inhibitor). It is to be understood that such combination therapy constitutes a further aspect of the invention.

According to a further aspect of the present invention there is provided a method for treating a MHC class II dependent T-cell mediated autoimmune or inflammatory disease, for example one or more of the diseases or medical conditions referred to herein, which

comprises administering to a warm-blooded mammal (including man) in need of such treatment an effective amount of a peptide compound of formula I, or a pharmaceutically acceptable salt thereof. The invention also provides the use of a chemical compound of formula I, or an amide thereof, or a pharmaceutically acceptable salt thereof in the production of a novel medicament for use in the treatment of a MHC class II dependent T-cell mediated autoimmune or inflammatory disease.

In addition to their aforesaid use in therapeutic medicine in humans, the peptide compounds of formula I are also useful in the veterinary treatment of similar conditions affecting commercially valuable warm-blooded animals, such as dogs, cats, horses and cattle. In general for such treatment, the peptide compound of the formula I will be administered in an analogous amount and manner to those described above for administration to humans. The chemical compounds of formula I are also of value as pharmacological tools in the development and standardisation of test systems for the evaluation of the effects of MHC class II molecules in laboratory animals such as cats, dogs, rabbits, monkeys, rats and mice, as part of the continuing search for new and improved therapeutic agents, or as diagnostic reagents.

The invention will now be illustrated by the following non-limiting Examples in which, unless otherwise stated:-

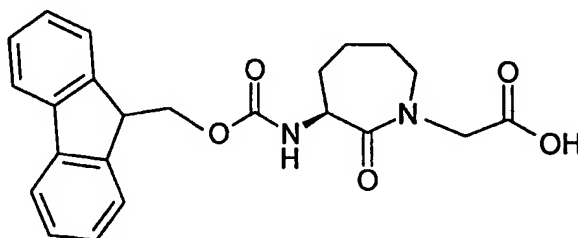
- (i) concentrations and evaporations were carried out by rotary evaporation in vacuo;
- (ii) operations were carried out at room temperature, that is in the range 18-26°C;
- (iii) yields, when given, are intended for the assistance of the reader only and are not necessarily the maximum attainable by diligent process development;
- (iv) the following abbreviations are used:
Phv = 5-phenylvaleryl; Boc = tert-butoxycarbonyl; Pbf = 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulphonyl; DMF = N,N-dimethylformamide; HOBT = 1-hydroxy-benzotriazole; Met = methionine; Fmoc = 9-fluorenylmethoxycarbonyl; Fmoc-Pip-OH = N-(9-fluorenylmethoxycarbonyl)piperidine-4-carboxylic acid; Fmoc-Papa-OH = 4-[N-(9-fluorenylmethoxycarbonyl)amino]phenylacetic acid; Cbz = benzyloxycarbonyl; THF = tetrahydrofuran; DMSO = dimethylsulfoxide; HBTU = 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluroniumhexafluorophosphate; DIPEA = diisopropylethylamine; TFA = trifluoroacetic acid; HPLC = high pressure liquid chromatography; MPLC = medium pressure liquid chromatography; and RP-HPLC = reverse phase high pressure liquid chromatography;

- (v) flash chromatography and MPLC was performed on Merck Kieselgel 60 (Art No. 9385) obtained from E Merck, Darmstadt, Germany; and
- (vi) ¹H NMR spectra were determined at 200 Mhz in CDCl₃ or d₆-dimethylsulphoxide (d₆-DMSO) using tetramethylsilane (TMS) as an internal standard, and are expressed as chemical shift (delta) values in parts per million relative to TMS using conventional abbreviations for designation of major peaks: s, singlet; m, multiplet; t, triplet; br, broad; d, doublet.
- 5

Examples

Example 1 Phv-Arg-Ala-Ala-Iib-Ala-Ala-Ala-Papa-NH₂ (SEQ ID NO:1)

5 **1.1** **Preparation of (3S)-3-(9-fluorenylmethoxycarbonylamino)-2-oxoperhydroazepine-1-acetic acid (Fmoc-Iib-OH).**



10

- (a) (3S)-3-Amino-ε-caprolactam (25g) and triethylamine (19.7g) were dissolved in THF (200ml) and cooled to 5°C. Benzyl chloroformate (33g) in THF (50ml) was added dropwise over 30 minutes. The reaction mixture was stirred at ambient temperature for 18 hours and then poured into water (500ml). The reaction mixture was extracted with ethyl acetate (3x100ml). The combined extracts were dried (MgSO₄) and evaporated. The residue was trituated with ether (40ml) and filtered to give 20g of (S)-3-benzyloxycarbonylamino-ε-caprolactam as a white solid; NMR (d₆-DMSO): 1.1-2(m, 6H), 2.9-3.2(m, 2H), 4.1-4.25 (m, 1H), 5.0 (s, 2H), 7.25-7.4 (m, 5H), 7.75 (t, 1H).
- 15
- (b) A mixture of sodium hydride (2g) in DMF (100ml) was cooled under a stream of argon to 0°C. (3S)-3-Benzyloxycarbonylamino-ε-caprolactam (10g) was added portionwise over 20 minutes at such a rate to keep the reaction temperature below 5°C. Stirring was continued at 0°C for 40 minutes and tert-butyl bromo acetate (8.2g) was then added dropwise over 10 minutes. The reaction mixture was stirred at 0°C for 1 hour and for a further 18 hours at ambient temperature. The reaction mixture was poured into water (600ml) and extracted with ethyl acetate (6x75ml). The combined extracts were washed with water (3x100ml), dried (MgSO₄) and evaporated. The residue was purified by MPLC on silica eluting with 20% ethyl
- 20
- 25

acetate/dichloromethane to give tert-butyl (3S)-3-benzyloxycarbonylamino-2-oxoperhydroazepine-1-acetate (15g) as a clear oil; NMR (d_6 -DMSO): 1.4 (s, 9H), 1.5-1.9 (m, 6H), 3.5-3.65 (m, 1H), 3.9-4.15 (m, 2H), 4.4 (m, 1H), 5.0 (s, 2H), 7.1 (d, 1H), 7.35 (m, 5H); mass spectroscopy, m/e (ES+) 377 (MH⁺).

5

(c) tert-Butyl (3S)-3-benzyloxycarbonylamino-2-oxoperhydroazepine-1-acetate (15g) was dissolved in ethanol (150ml) and purged with argon. 10% palladium on carbon (1.5g) was added and the flask evacuated and filled with hydrogen from a balloon. The reaction mixture was stirred at ambient temperature for 4 hours. The reaction mixture was then purged with argon and filtered through diatomaceous earth. The filtrate was evaporated to give tert-butyl (3S)-3-amino-2-oxoperhydroazepine-1-acetate (7.7g) as a viscous oil; NMR (CDCl₃): 1.45 (s, 9H), 1.55-2.05 (m, 6H), 3.2-3.3 (d, 1H), 3.55-3.75 (two overlapping doublets, 2H), 3.95-4.25 (q, 2H); mass spectroscopy, m/e (ES+) 243.2 (MH⁺).

15 (d) A solution of tert-butyl (3S)-3-amino-2-oxoperhydroazepine-1-acetate (7g) in THF (50ml) was added to a solution of sodium carbonate (3g) in water (30ml). A solution of N-(9-fluorenylmethoxycarbonyloxy)succinimide (9.7g) in THF (100ml) was then added dropwise over 30 minutes with stirring. The reaction mixture was stirred at ambient temperature for a further 30 minutes. Water (200ml) was added and the reaction mixture extracted with ethyl acetate (3x100ml). The combined extracts were washed with brine (100ml), dried (MgSO₄) and evaporated. The residue was purified by MPLC on silica, eluting initially with dichloromethane and gradually increasing to 15% ethyl acetate/dichloromethane to give tert-butyl (3S)-3-(9-fluorenylmethoxycarbonylamino)-2-oxoperhydroazepine-1-acetate (10.9g) as a clear oil; NMR (d_6 -DMSO): 1.45 (s, 9H), 1.5-2.15 (m, 6H), 3.1-3.25 (m, 1H), 3.6-3.75 (m, 1H), 4.0-4.5 (m, 5H), 6.25 (d, 1H), 7.25-7.45 (m, 4H), 7.6 (d, 2H), 7.75 (d, 2H); mass spectroscopy, m/e (ES+) 465.2 (MH⁺).

25 (e) tert-Butyl (3S)-3-(9-fluorenylmethoxycarbonylamino)-2-oxoperhydroazepine-1-acetate (10.5g) was dissolved in dichloromethane (30ml) and trifluoroacetic acid (20ml) was added. The reaction mixture was stirred at ambient temperature for 18 hours. Dichloromethane (100ml) was added and the reaction mixture was washed with water (4x100ml), dried (MgSO₄) and evaporated. The residue was purified by MPLC on silica

eluting with 25% ethylacetate/dichloromethane, followed by ethyl acetate, to give an oil.

Trituration with isohexane gave a white foam which was filtered and dried under vacuum at 60°C to give (3S)-3-(9-fluorenylmethoxycarbonylamino)-2-oxoperhydroazepine-1-acetic acid

(5.5g); NMR (d_6 -DMSO): 1.4-1.95 (m, 1H), 3.15-3.4 (m, 1H), 3.55-3.7 (m, 1H), 3.9-4.2 (m, 2H), 4.25-4.45 (m, 2H), 7.15 (d, 1H), 7.25-7.45 (m, 4H), 7.75 (m, 2H), 7.85 (d, 2H); mass spectrometry, m/e (ES-) 407.1 (M-H).

1.2 Phv-Arg-Ala-Ala-IIb-Ala-Ala-Ala-Papa-NH₂

- 10 The peptide was constructed by solid phase peptide synthesis (using an automated synthesiser (ABI 430A)) starting with Fmoc-Rink Amide MBHA resin (Novabiochem, 1.587g, 1mmol). The coupling and deprotection steps were carried out in the following manner. The Fmoc protecting group was removed by treatment with a 20% solution of piperidine in DMF (two treatments; the first for 3 minutes and the second for 12 minutes; both with 10-20 ml of solution). The resin was washed with DMF (10 x 10-20 ml). The carboxylic acid (1mmol) was activated with HBTU (1 equivalent), HOBT (1 equivalent) and DIPEA (2 equivalents) in DMF for approximately 11 minutes before transfer to the resin. The acylation was carried out for approximately 60 minutes and then the resin was washed with DMF (10 x 10-20 ml). The acylation and washings were repeated (a total of two couplings for each carboxylic acid).
- 20 This cycle of Fmoc deprotections and couplings was repeated for each of the carboxylic acids.

The following acids were used in the following order:

	Fmoc-Papa-OH	373mg, 1mmol	(two couplings)
	Fmoc-Ala-OH	312mg, 1mmol	(two couplings)
25	Fmoc-Ala-OH	312mg, 1mmol	(two couplings)
	Fmoc-Ala-OH	312mg, 1mmol	(two couplings)
	Fmoc-IIb-OH	407mg, 1mmol	(two couplings)
	Fmoc-Ala-OH	312mg, 1mmol	(two couplings)
	Fmoc-Ala-OH	312mg, 1mmol	(two couplings)
30	Fmoc-Arg(Pbf)-OH	752mg, 1mmol	(two couplings)
	5-Phenylvaleric acid	178mg, 1mmol	(two couplings)

The completed resin was collected, washed with methanol and dried for 16 hours in vacuo at 45°C to give the peptide resin (2.439g). The peptide was cleaved from the resin and deprotected by treatment with TFA (15ml), water (2ml) and triethylsilane (300µl) for 90 minutes at ambient temperature. The mixture was filtered and the resin was washed with TFA (3 x 5ml). The combined filtrate and washings were evaporated and the residue was dissolved in a mixture of water and acetonitrile and freeze-dried. The peptide was partially purified by dividing into 5 portions and carrying out preparative RP-HPLC (Vydac 201HS1022 column, 250mm x 22mm) on each portion, loading the crude material in 5ml of 20% acetonitrile/water with 200 microlitres of acetic acid. Elution was carried out using a gradient of acetonitrile-water containing 0.1% TFA (15-45% acetonitrile) over 60 minutes at a flow rate of 12ml/minute. The fractions containing product were combined and freeze dried to give a white solid (700mg).

The product was then further purified and converted to the acetate salt by preparative RP-HPLC (in 3 portions) eluting with a gradient of buffer A (0.1M ammonium acetate (AR, Fisons) in water) and buffer B (0.1M ammonium acetate in 60% aqueous acetonitrile), using a 35-60% buffer B gradient over 60 minutes at 12ml/min. Pure fractions were combined and freeze-dried and the freeze-drying was repeated from water until a constant weight was obtained (476mg).

The product was characterised by HPLC, mass spectroscopy and amino acid analysis. RP-HPLC (Vydac C18 column, 201HS, 4.6 x 250mm, eluting with acetonitrile and water containing 0.1% TFA, using a 10-40% acetonitrile gradient over 40 minutes, flow rate 1.2ml/minute) retention time = 27.2 minutes.

Mass spectroscopy. m/e (ES+) 990.4 (MH⁺)

Amino acid analysis (acid hydrolysis over 24 hours using a solution of 6N HCl containing 1% Phenol at 130°C) gave Arg 1.16, Ala 4.88, Ile present.

Fmoc-Papa-OH was obtained by an analogous method to that described in E. Atherton and R. C. Sheppard ("Solid phase peptide synthesis: a practical approach", IRL press, 1989, page 51) for *N*-Fmoc-L-methionine:

Fmoc-Papa-OH: NMR (DMSO-d₆) 3.5 (s, 2H), 4.25 (t, 1H), 4.5 (d, 3H), 7.1 (d, 2H), 7.4 (m, 6H), 7.75 (d, 2H), 7.9 (d, 2H), 9.6 (s, 1H); mass spectrometry m/e (ES-) 372.1 (M-H)⁻.

Example 2 Phv-Arg-Ala-Ala-Ala-Thr-Iib-Ala-Papa-NH₂ (SEQ ID NO:2)

The first part of the synthesis was carried out manually by Fmoc solid phase synthesis starting with Fmoc Rink Amide MBHA Resin (Novabiochem, 0.50g, 0.25mmoles) in a Bond Elut
5 tube (Varian, 15ml, fitted with a filter in the bottom).

- (a) The resin was deprotected using a 20% solution of piperidine in DMF (two treatments with 5ml for 10 minutes each). After deprotection the resin was thoroughly washed with DMF (5 x 10ml).
- 10 (b) Acylation was carried out by addition of a solution of Fmoc-Papa-OH (353mg, 1mmol), DMF(1.5ml), HOBT (135mg, 1mmol), DIPEA (261μL, 1.5mmol) and HBTU (379mg, 1mmol). The coupling was left for approximately 30 minutes, washed with DMF (5x10ml) and a small portion of the resin checked for completion of acylation using the Kaiser
15 test (E. Kaiser et al, (1970), Anal. Biochem. 34, 595).

The above deprotection (a) and coupling cycles (b) were repeated using Fmoc-Ala-OH, Fmoc-Iib-OH (triple couple) and Fmoc-Thr(OtBu)-OH (double couple). The remainder of the sequence was constructed on an automated system (ACT 357) using Fmoc-Ala-OH,
20 Fmoc-Arg(Pbf)-OH and 5-Phenylvaleric acid at the appropriate stage and using similar coupling chemistry. The peptide was cleaved from the resin, deprotected and purified using a similar procedure to that described in Example 1.2 (but without using the ammonium acetate buffered preparative HPLC procedure).

The product was characterised by HPLC, mass spectroscopy and amino acid analysis.
25 RP-HPLC (Vydac C18 column, 218TP54, 4.6 x 250mm, eluting with acetonitrile and water containing 0.1% TFA, using a 20-50% acetonitrile gradient over 30 minutes, flow rate 1.0ml/minute) retention time = 17.01 minutes.

Mass spectrometry, m/e (ES+) 1020.6 (MH⁺).

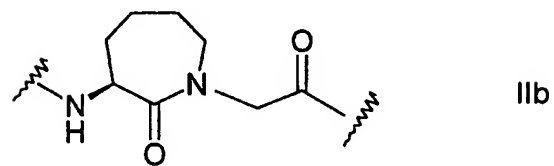
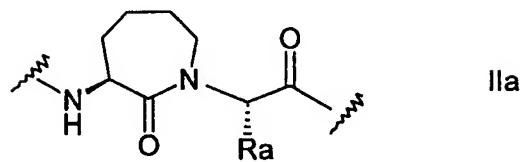
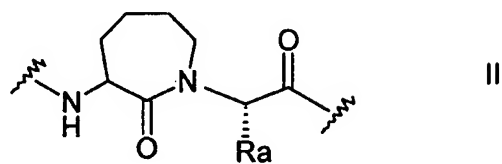
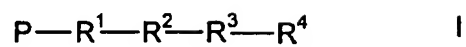
Amino acid analysis (acid hydrolysis over 24 hours using a solution of 6N HCl containing 1%
30 phenol at 130°C) gave Arg 1.00, Ala 4.12, Iib present, Thr 0.88.

Example 3

The compounds of the invention may be administered for therapeutic or prophylactic use to warm-blooded animals such as man in the form of conventional pharmaceutical compositions,
5 a typical example of which includes the following:-

Injectable Solution

0.01 to 100 mg of active ingredient is dissolved in up to 2 ml of an aqueous injection vehicle to give a concentration of active ingredient between 0.01 to 100 mg/ml. The aqueous
10 injection vehicle is buffered to a pH between 5 and 8 using a pharmaceutically acceptable buffer (for example, phosphate, or acetate) and contains a pharmaceutically acceptable tonicity adjustment agent (for example, sodium chloride or dextrose) added to achieve isotonicity. The vehicle may optionally also contain other pharmaceutically acceptable excipients such as solubilising agents (for example, DMSO, ethanol, propylene glycol or
15 polyethylene glycol) preservatives and antioxidants. The active ingredient may typically be an Example described hereinbefore and may conveniently be present as a pharmaceutically acceptable salt.

CHEMICAL FORMULAE

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: ZENECA LIMITED
(B) STREET: 15 STANHOPE GATE
(C) CITY: LONDON
(E) COUNTRY: UNITED KINGDOM
(F) POSTAL CODE (ZIP): W1Y 6LN
(G) TELEPHONE: 0171 304 5000
(H) TELEFAX: 0171 304 5151
(I) TELEX: 0171 834 2042

(ii) TITLE OF INVENTION: PEPTIDE COMPOUNDS

(iii) NUMBER OF SEQUENCES: 2

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(vi) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: GB 9621836.7
(B) FILING DATE: 19-OCT-1996

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

(A) NAME/KEY: Peptide
(B) LOCATION:1
(D) OTHER INFORMATION:/product= "OTHER"
/note= "5-Phenylpentanoyl-Arg"

(ix) FEATURE:

(A) NAME/KEY: Peptide
(B) LOCATION:4
(D) OTHER INFORMATION:/product= "OTHER"
/note= "2-((S)-3-amino-2-oxoperhydroazepin-1-yl)acetyl-Ala"

(ix) FEATURE:

(A) NAME/KEY: Peptide
(B) LOCATION:6
(D) OTHER INFORMATION:/product= "OTHER"
/note= "Ala-4-aminophenylacetamide"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Xaa Ala Ala Xaa Ala Xaa
1 5

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION: 1
- (D) OTHER INFORMATION: /product= "OTHER"
/note= "5-Phenylpentanoyl"

(ix) FEATURE:

- (A) NAME/KEY: Peptide
- (B) LOCATION: 6
- (D) OTHER INFORMATION: /product= "OTHER"
/note=
"2-((S)-3-amino-2-oxoperhydroazepin-1-yl)acetyl-Ala-4-aminophenylacetamide"

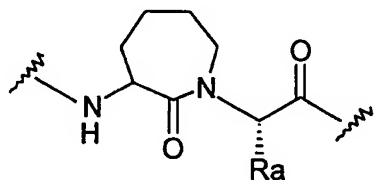
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Xaa Ala Ala Ala Thr Xaa
1 5

CLAIMS

What we claim is:-

1. A peptide compound of the formula I, $P-R^1-R^2-R^3-R^4$, or a pharmaceutically acceptable salt thereof, wherein
- P is a hydrophobic residue;
- R^1 is a sequence of 5 L-amino acids and R^3 is a single L-amino acid; or
- R^1 is a sequence of 3 L-amino acids and R^3 is a sequence of 3 L-amino acids;
- R^2 is a group of formula II



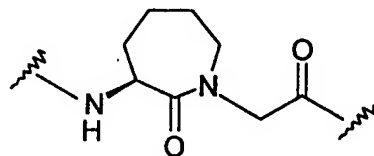
II

- in which R_a is independently selected from hydrogen and (1-4C)alkyl; and
- R^4 is OH, NH_2 or NR^cR^d wherein R^c is selected from (1-4C)alkyl, 2-carbamoylcyclopentyl, 2-pyridylmethyl, 4-carbamoylcyclohexyl, 4-carbamoylcyclohexylmethyl, 3-carbamoylphenyl, 4-carbamoylphenyl, 4-(carbamoylmethyl)phenyl, 4-(carboxymethyl)phenyl, 2-morpholinoethyl and a group of the formula $-A^1-G^1$ in which A^1 is (3-7C)alkylene or A^1 is selected from
- (1) a group of the formula $-A^2-B^2-$ in which A^2 is *p*-phenylene or 1,4-cyclohexylene and B^2 is (1-4C)alkylene or A^2 is methylene and B^2 is *p*-phenylene or 1,4-cyclohexylene; and
- (2) a group of the formula $-A^3-B^3-C^3-$ in which A^3 is methylene, B^3 is *p*-phenylene or 1,4-cyclohexylene and C^3 is (1-3C)alkylene; and
- G^1 is a group of the formula $-N=C[N(R^p)_2]_2$ in which each R^p is independently selected from hydrogen, methyl, ethyl and propyl; and R^d is hydrogen or (1-4C)alkyl; or
- R^4 is 1-piperazinyl, 4-methyl-1-piperazinyl, 4-amidino-1-piperazinyl, 4-(2-(2-hydroxyethoxy)ethyl)-1-piperazinyl, 1-piperidyl or 4-substituted-1-piperidyl wherein the 4-substituent is selected from carboxy, carbamoyl, N-(2-aminoethyl)carbamoyl and N-(4-aminobutyl)carbamoyl; or

R⁴ is a sequence of 1 to 6 amino acids or an amide thereof.

2. A peptide compound, or pharmaceutically acceptable salt thereof, as claimed in claim 1 in which P is a hydrophobic aliphatic, aromatic, heteroaromatic or mixed aliphatic/aromatic
5 or aliphatic/aromatic or aliphatic/heteroaromatic organic group of 5 to 20 carbon atoms and 1,2 or 3 heteroatoms selected from oxygen, sulphur and nitrogen.
3. A peptide compound, or a pharmaceutically acceptable salt thereof, as claimed in claim 1 or 2 wherein R¹ is a sequence of 5 L-amino acids represented as AA1-AA2-AA3-
10 AA4-AA5 in which
AA1 is selected from Ala, Ile, Tyr, Val, Glu, Lys, Arg, Gly, Gap, GapMe₄ and 3,3,3-trifluoroalanine;
AA2 is selected from Ala, Lys, Glu, Sar, Val, Arg, Gly, Pro, Ile, Tic, 3,3,3-trifluoroalanine and N⁶-diethylLys;
15 AA3 is selected from Ala, His, Gln, Val, Thr, Glu, Gly, Asp, Asn and N³-diethylDap;
AA4 is selected from Ala, Lys, Asn, Arg, Thr, Glu, Sar, Gly, Pro, His and N⁶-diethylLys; and
AA5 is selected from Thr, Val, Ala, Gly, Dap, Dab, Pro, Hyp, Asn and N³-diethylDap; and
R³ is a single amino acid selected from Ala, Gly, Dap, azaalanine and azaglycine.
- 20 4. A peptide compound, or a pharmaceutically acceptable salt thereof, as claimed in claim 1 or 2 wherein R¹ is a sequence of 3 L-amino acids represented as AA1-AA2-AA3 in which
AA1 is selected from Ala, Ile, Tyr, Val, Glu, Lys, Arg, Gly, Gap, GapMe₄ and 3,3,3-trifluoroalanine;
25 AA2 is selected from Ala, Lys, Glu, Sar, Val, Arg, Gly, Pro, Ile, Tic, 3,3,3-trifluoroalanine and N⁶-diethylLys; and
AA3 is selected from Ala, His, Gln, Val, Thr, Glu, Gly, Asp, Asn and N³-diethylDap; and R³ is selected from a sequence of 3 L-amino acids represented as AA6-AA7-AA8 in which
AA6 is selected from Gly, Leu, Lys, Ala, Pro, Glu, Sar, His and Dap;
30 AA7 is selected from Pro, Ala, Lys, Arg, Glu, Sar, Gly, Oic and Dic; and
AA8 is selected from from Ala, Gly, Dap, azaalanine and azaglycine.

5. A peptide compound, or pharmaceutically acceptable salt thereof, as claimed in any preceding claim wherein R^2 is a group of the formula IIb



IIb

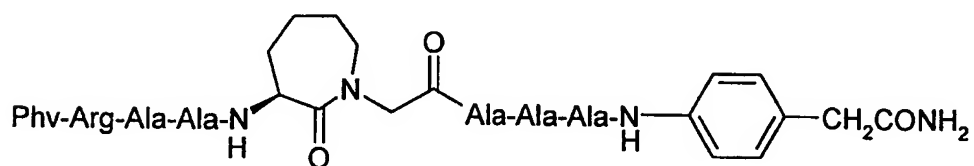
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6. A peptide compound, or a pharmaceutically acceptable salt thereof, as claimed in any preceding claim wherein hydrophobic group P is 5-phenylvaleryl.

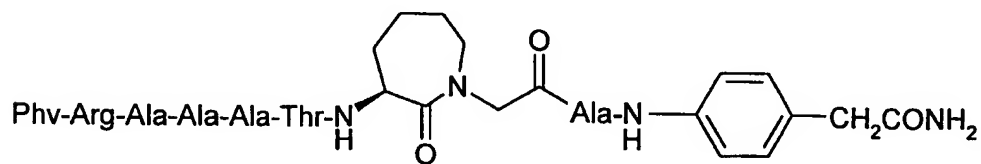
7. A peptide compound, or a pharmaceutically acceptable salt thereof, as claimed in any preceding claim wherein R^4 is 4-carbamoyl-1-piperidyl,
10 4-(carbamoylmethyl)anilino or 4-(2-guanidinoethyl)anilino.

8. A peptide compound as claimed in claim 1, or a pharmaceutically acceptable salt thereof, which is selected from

15



and



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or a pharmaceutically acceptable salt thereof, in which Phv represents a 5-phenylvaleryl group.

- 5 9. A pharmaceutical composition which comprises a peptide compound of the formula I, or a pharmaceutically acceptable salt thereof, as claimed in any preceding claim, in association with a pharmaceutically acceptable diluent or carrier.
- 10 10. A process for the manufacture of a peptide compound, or a pharmaceutically acceptable salt thereof, as claimed in claim 1, comprising sequentially coupling in the appropriate order suitably protected amino acids or sequences of two or more suitably protected amino acids, a suitably protected group of the formula H-II-OH and optionally a suitably protected group of the formula H-R⁴, followed by optional functional group modification of the N-terminal amino group to introduce a hydrophobic group P, and removal
15 of any remaining protecting groups and any solid support.
11. A method for treating a MHC class II dependent T-cell mediated autoimmune or inflammatory disease which comprises administering to a warm-blooded mammal in need of such treatment an effective amount of a peptide compound of formula I, or a pharmaceutically
20 acceptable salt thereof, as claimed in claim 1.
12. A method as claimed in claim 11 for treating rheumatoid arthritis or multiple sclerosis.
13. The use of a peptide compound of formula I, or a pharmaceutically acceptable salt
25 thereof, as claimed in any of claims 1 to 8, in the production of a novel medicament for use in the treatment of a MHC class II dependent T-cell mediated autoimmune or inflammatory disease.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 97/02837

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C07K/06 A61K38/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 92 02543 A (CYTEL CORP ;SANDOZ LTD (CH); SANDOZ AG (DE)) 20 February 1992 cited in the application see page 7, paragraph 4 see the whole document ---	1-7,9-13
P,X	WO 97 31023 A (ZENECA LTD ;COTTON RONALD (GB); EDWARDS PHILIP NEIL (GB); LUKE RIC) 28 August 1997 see the whole document ---	1-13
A	EP 0 644 197 A (STERLING WINTHROP INC) 22 March 1995 see page 2, line 19-20 see page 4, line 25-30 ---	
-/--		

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

Z document member of the same patent family

Date of the actual completion of the international search

27 January 1998

Date of mailing of the international search report

13. 02.98

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Cervigni, S

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 97/02837

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4 474 778 A (GORDON ERIC M ET AL) 2 October 1984 see examples 1-37 ---	10
A	BROWN J H ET AL: "THREE-DIMENSIONAL STRUCTURE OF THE HUMAN CLASS II HISTOCOMPATIBILITY ANTIGEN HLA-DR1. " NATURE, vol. 364, 1 July 1993, pages 33-39, XP002046347 see page 38, column 1, paragraph 1 -----	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 97/02837

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
see FURTHER INFORMATION sheet PCT/ISA/210
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this International application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Remark : Although claims 11 and 12 are directed to a method of treatment of the human/animal body , the search has been carried out and based on the alleged effects of the compound/composition.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 97/02837

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9202543 A	20-02-92	AU 8305491 A US 5679640 A	02-03-92 21-10-97
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US 4474778 A	02-10-84	AU 3522084 A EP 0142335 A JP 60115565 A	16-05-85 22-05-85 22-06-85
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